PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:		10/080,713	Confirmation No.: 9155
Appellants	:	Alan Colman et al.	
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TC/A.U.	:	1600/1632	
Examiner	:	Thaian N. Ton	
Title	:	Method of Preparing a Somatic Cell for Nuclear Transfer	
Docket No.	:	10758.105015	
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APPEAL BRIEF

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L. Claims 82, 113, 121 and 122 are fully enabled

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(viii) CLAIMS APPENDIX

(ix) EVIDENCE APPENDIX

A. Declaration of David L. Ayares, Ph.D. dated June 23, 2005

B. Declaration of Jorge A. Piedrahita, Ph.D. dated April 21, 2006

(i) REAL PARTIES IN INTEREST

The real party in interest in this case is the assignee, Revivicor, Inc, and its licensee, Zimmer, Inc.

(ii) RELATED APPEALS AND INTERFERENCES

none

(iii) STATUS OF CLAIMS

Claim 62, 63, 65, 66, 70-73, 75-79, 82, 87-90, 99-100, 102-110, 113, 118-125, 131 and 133 are pending and on appeal.

(iv) STATUS OF AMENDMENTS

The claims were amended in an amendment filed February 16, 2007. In the Advisory Action mailed March 21, 2007, the Examiner stated that this amendment would be entered. An appendix sets forth the claims on Appeal.

(v) SUMMARY OF CLAIMED SUBJECT MATTER

The claims are grouped as follows: claim 131; claim 133; claims 62 & 90; claim 63; claims 65 and 99; claims 66 and 100; claims 70-73 & 102-105; claims 75 & 106; claims 76-79 & 107-110; claims 82, 113 & 121-122; claims 123 & 124; claim 125; claims 87 & 118; claims 88-89 & 119-120. The claims do not stand or fall together.

Claim 131 recites a method for producing a non-human transgenic mammal by modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event; and accomplishing successful nuclear transfer to produce the non-human transgenic mammal.

Claim 133 recites a method for producing a non-human transgenic mammal by modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event; transferring the modified nuclear genome of the somatic cell to an oocyte, two cell embryo or zygote, which is capable of

producing a viable nuclear transfer unit; activating the nuclear transfer unit; transferring the embryo to a surrogate mother and allowing the embryo to mature.

Claims 62 and 90 recite a method for producing a non-human transgenic mammal by modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event; transferring the modified nuclear genome of the somatic cell to an oocyte, two cell embryo or zygote, which is capable of producing a viable nuclear transfer unit; activating the nuclear transfer unit; transferring the embryo to a surrogate mother and allowing the embryo to develop to term to produce a non-human transgenic mammal. Claim 90 also includes the step of breeding the transgenic mammal to produce transgenic offspring from the transgenic mammal.

Claim 63 further limits the method of claim 62 by specifying that the transgenic mammal is a transgenic sheep, cattle or pig.

Claims 65 and 99 further limit the method of claims 62 and 90, respectively, by specifically reciting that the genetic targeting event results in removal of a gene, modification of a gene, upregulation of a gene, gene replacement or transgene placement.

Claims 66 and 100 further limit the method of claims 62 and 90, respectively, by specifically reciting that the genetic targeting event results in inactivation of a gene.

Claims 70-73 & 102-105 further limit the method of claims 62 and 90, respectively, by reciting that the modification places a promoter adjacent to an endogenous gene and further, the identity and/or activity of the promoter.

Claims 75 and 106 further limit the method of claims 62 and 90, respectively, by reciting that the modification places a marker gene at the endogenous locus in the nuclear genome.

Claim 76-79 and 107-110 further limit the method of claims 75 and 106, respectively, by reciting that the marker gene confers resistance to neomycin, G418, hygromycin, zeocin, blasticidin and/or histidinol; the marker gene is HPRT, gpt, a visible marker gene and/or a gene that can be detected with a single chain antibody/hapten system; and/ or that the visible marker gene is GFP.

Claims 82, 113, 121 and 122 further limit claims 62 and/ or 90 by reciting that the genetic targeting event is mediated by lipofection, electroporation or transfection.

Claims 123 and 124 further limit the method of claims 66 and 99, respectively, by reciting that the gene that is inactivated is α-1,3 galactosyltransferase.

Claim 125 further limits the method of claims 62 or 90, by reciting that the endogenous locus is an immunoglobulin gene.

Claims 87 and 118 limit the method of claims 62 and 90, respectively, by reciting that the somatic cell is an epithelial cell, a fibroblast cell or an endothelial cell.

Claims 88-89 & 119-120 limit the method of claims 62 and 90, respectively, by reciting that the somatic cell is a G_0 cell or a G_0 cell obtained by serum starvation of a somatic cell.

(vi) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

112 1st paragraph, enablement

(vii) ARGUMENTS

A. Summary

This section provides an overview of the specific rejections and arguments made in response thereto, which are detailed further below.

The present application describes and claims a method for the targeted, genetic modification of somatic cells followed by successful nuclear transfer. It combines two well-known techniques, homologous recombination and nuclear transfer, previously thought not to be amendable to successful combination by those of skill in the respective arts. It is a basic invention that has been widely recognized as groundbreaking in the relevant scientific literature.

The Examiner has rejected all pending claims as non-enabled. A brief review of the file history reveals that the Examiner has objected to enablement on two levels. First, to the enablement of the two known techniques, homologous recombination and nuclear transfer, independent of their combination in the present invention. Second, to the invention as a whole, i.e., the combination of the two techniques according to the present invention.

The Examiner has argued that homologous recombination in somatic cells requires undue experimentation. Appellants have obviously objected. Gene targeting techniques are well known and have been used in the art for over 20 years. The

techniques used to target genes are the same, regardless of whether the cell is a stem cell, somatic cell or any other cell. In 1999, only a few experiments in somatic cells had been reported and these were characterized by relatively low efficiencies. Most people skilled in the art instead used embryonic stem (ES) stem cells. Appellants took an interest in somatic cells, however, and have successfully shown (in the pending application) that homologous recombination can, in fact, be carried out at relatively high frequencies in somatic cells, frequencies at least equal to that the frequencies achieved in ES cells. Put another way, Appellants have shown that somatic cells, never popular for homologous recombination because of early work associated with unacceptably low frequencies, are equally suited to gene targeting as the very popular, commonly used ES cells. The Examiner has cited references in support of non-enablement that describe the low frequencies characteristic of the early work. Appellants don't dispute that was, in fact, the state of the art prior to the present invention, but submit that the Appellants data directly contradicts any belief that somatic cells aren't equally useful for homologous recombination. Appellants have further established, including through Declarations, that methods for screening for homologous recombination events are routine.

With respect to nuclear transfer, the Examiner has argued that the technique is inefficient in producing live born animals, and thus non-enabled. To support this conclusion, the Examiner has cited post-filing publications that focus on possible ways to improve the overall efficiency of the cloning process and/or analyze the underlying molecular mechanisms. Appellants submit that the observation that a process could be more efficient provides inadequate basis to conclude that the process in non-enabled, given that the low efficiencies associated with nuclear transfer are acceptable in the cloning art. In general, Appellants aren't interested in ways to improve the cloning process or further understanding the molecular mechanisms that underlie the process. The Appellants have established a successful business model that employs the cloning process as it has existed for the last ten years, with characteristically low efficiencies, and have successfully cloned hundreds of animals (including genetically modified animals).

While the Examiner has characterized inefficiency as unpredictability, Appellants submit that the two concepts are distinguishable. It's possible for a method to be inefficient, but predictably so. Put another way, one skilled in the art would not know if

any given attempt will work, but one skilled in the art would know that within a set number of attempts, a certain percentage will work. If that percentage is acceptable within the art-- and it is as evidenced by the record--- that amount of experimentation should not be deemed "undue." In cloning, the inefficiency objected to by the Examiner is acceptable, predictable and informs the entire art, as described in detail in the Declaration submitted during prosecution. These Declarations describe the common and accepted practice within the art, where technicians regularly transfer large number of embryos to achieve live births. The Examiner has not provided any evidence to rebut the Appellant's evidence as to the amount of experimentation acceptable in the art.

With respect to the invention as a whole, the Examiner has provided no evidence to support lack of enablement. Rather, in response to the Appellants arguments and Declarations in support of the enablement of homologous recombination and nuclear transfer as independent techniques, the Examiner has cited the non-enablement of the invention *as a whole.* In fact, there is no evidence available of which the Appellants are aware or that the Examiner has cited that would support of lack of enablement for the invention as a whole. To the contrary, the Appellants know only of reports of success of the method as carried out by at least nine independent groups since the publication of the pending application. These nine groups have successfully targeted five different genes in three different species using fifteen different targeting constructs. Appellants maintain that the enablement of the invention as a whole, rather the individual methods combined to arrive at the invention, is in fact the proper focus for the enablement analysis.

Despite the arguments presented to date, as further supported by two Declarations of those of skill in the art (including a disinterested third party), the Examiner argues that the pending claims are non-enabled. Appellants submit that the Examiner has failed to meet her burden to prove that the invention is non-enabled, and has failed to consider the evidence as a whole.

Each separate rejection under § 112, first paragraph, is discussed below.

B. Legal Standard for Enablement

35 U.S.C. 112, first paragraph, provides:

"The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same,"

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under 35 U.S.C. § 112, first paragraph, as whether at the time of filing one skilled in the art could make and use the claimed invention from the disclosures in the patent, coupled with information known in the art, without undue experimentation. (See, e.g., Genentech, Inc. v. Novo Nordisk A/S, 108 F.3d at 165 (quoting In re Wright, 999 F.2d 1557, 1561 (Fed. Cir. 1993)); See also In re Fisher, 427 F.2d at 839; United States v. Telectronics, Inc., 857 F.2d 778 (Fed. Cir. 1985); and Manual of Patent Examination Procedure (MPEP) § 2164.01).

Undue experimentation is evaluated by the factors laid out by the Federal Circuit in Wands. 858 F.2d 731, 737 (Fed. Cir. 1988). These factors include but are not limited to (i) the breadth of the claims; (ii) the nature of the invention; (iii) the state of the prior art; (iv) the level skill in the art, as determined by reference to a person of ordinary skill; (v) the level of predictability in art; (vi) the amount of direction provided by the inventor; the existence of working examples; and (vii) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. Id.

"It is well-established that the amount of experimentation that is considered 'undue' varies from one field to another." Id. Numerous decisions of this Board and other Courts speak specifically to the high degree of experimentation acceptable in connection with biological processes, cell cultures, gene therapy and other disciplines within the biotechnological arts. See, e.g., Ex Parte Barrett Rollins 2006 WL 2523796 (BPAI), In re Certain Limited-Charge Cell Culture Microcarriers, 221 USPQ 1165 (U.S. Intern. Trade Comm'n 1983), Johns Hopkins University v. Cellpro, Inc., 152 F.3d 1342, 1360 (Fed. Cir. 1998). "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the

experimentation should proceed." Wands, 858 F.2d at 737. A disclosure may be enabling even though a considerable amount of routine experimentation is required to practice the invention. *See* PPG Indus. Inc. v. Guardian Indus. Corp., 75 F.3d 1558, 1564 (Fed.Cir.1996).

It is the Examiner's burden to show non-enablement. MPEP § 2164.02. An invention is enabled unless the Examiner evidentiarily rebuts the objective truth of the statements made in the application. In re Marzocchi, 439 F.2d 220, 223 (CCPA 1971). [I]t is incumbent upon the Patent Office, whenever [an enablement] rejection ... is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." In re Wright, 999 F.2d 1557, 1561-62 (Fed. Cir. 1993). The MPEP expressly prohibits Examiners from rejecting applications based on personal opinion. MPEP § 2164.05. See Ex Parte Roland Stoughton 2006 WL 1665412 (BPAI). The Examiner is required to consider the original disclosure and all evidence in the record, and any conclusion of non-enablement must be based on the evidence as a whole. MPEP § 2164.01 (a).

The Board routinely overturns enablement rejections based on an Examiner's failure to produce evidence of non-enablement. See, e.g., Ex Parte Axel Kahn, 2006 WL 2523713 (BPAI); Ex Parte George Norbert Cox, 2006 WL 2851410 (BPAI); Ex Parte Roland Stoughton, 2006 WL 1665412 (BPAI); Ex Parte Nana K. Ayisi, 2006 WL 2822236 (BPAI); Ex Parte Barrett Rollins, 2006 WL 2523796 (BPAI), Ex Parte David B. Weiner, 2006 WL 2523787 (BPAI); Ex Parte Thomas E. Wagner, 2004 WL 4978893 (BPAI).

C. Claim 131 is fully enabled

Claim 131 is directed to a method for producing a non-human transgenic mammal by modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event, and accomplishing successful nuclear transfer to produce the non-human transgenic mammal.

This pending application is the first in the world to describe the specific modification of endogenous genes in somatic cells through a genetic targeting event followed by successful nuclear transfer. This invention was heralded in a full paper in

Nature magazine in 2000 (McCreath et al. (Nature 2000 Jun 29;405(6790):1066-9) and widely reported as a breakthrough in nuclear transfer and animal cloning.

In a *News and Views* article regarding this paper appearing in the same issue of Nature, it is stated that:

> Until now, no one had shown that it would be possible to specifically modify endogenous genes by cloning....This technique should provide a general way to produce specific genetic changes in several mammalian species. But the opportunities extend far beyond the optimal positioning of foreign genes....We are clearly at the dawn of a new era in mammalian genetic technology.

Suraokar and Bradley, *Nature* 405:1004-1005 (2000)).

At the time that the present application was filed, the two separate techniques of gene targeting and somatic nuclear transfer were well-established in the respective arts, as detailed further below. The present application differs from information known in the art not with regard to the individual techniques used, but rather in the success achieved by their combination. In essence, the present inventors achieved success and addressed a long-felt need by combining art-known methods that were, at the time of filing of the present application, thought not to be amenable to successful combination. It was an unexpected result that this combination would result in the production of viable cloned genetically modified animals.

The Examiner has maintained that the pending claims are not enabled. To briefly summarize the file history to date, as detailed further below, the Examiner has not provided any evidence to support lack of enablement of the invention as a whole per se, but rather has based the enablement rejections on references that the Examiner has cited as teaching that the nuclear transfer component or the homologous recombination component is unpredictable and/ or requires undue experimentation.

Appellants submit that as the two separate techniques were well known at the time of filing, there was no need to provide any further guidance other than what is in the present specification in order for the ordinarily skilled artisan to have been able to make and use the claimed invention. One of ordinary skill, reading the present specification in view of information known in the art, would not have had to resort to undue

experimentation in order to make and use the invention. Appellants now review the file history as it relates to specific rejections and arguments concerning enablement.

1. Homologous Recombination

Homologous recombination at a specified target in somatic cells does not require undue experimentation.

The Examiner has maintained the rejection in the 6/16/06 Final Office Action that one skilled in the art would be required to engage in undue experimentation to specifically target a particular gene in the genome of a somatic cell, modify this particular gene and then identify and select the recombinant cells.

As of the filing date of the present invention, gene targeting technology had been around for almost 20 years. In 1993, the techniques were sufficiently well established that a text book was published ("Gene Targeting: A Practical Approach. Alexandra L. Joyner, ed. Oxford University Press (1993); submitted as reference AS11 in Appellants Information Disclosure Stated filed 5/22/2002). The gene targeting technology is the same for all cells regardless of whether the cell is a somatic cell, a stem cell or any other cell.

Porter and Dallman's review "Gene Targeting: Techniques and Applications to Transplantation" in 1997 (submitted as reference AR22 in Appellants Information Disclosure Stated filed 5/22/2002) describes the state of gene targeting in *somatic* cells at the time the present application was filed. On page 1229, Porter and Dallman report that "although initial gene targeting experiments were carried out in cell lines derived from somatic tissues, efforts were quickly diverted to gene targeting in mouse embryonic stem cell." Only a few experiments reporting successful gene targeting in somatic cells had been carried out as of 1999, and the efficiencies were sufficiently low that the technique was not believed to be compatible with nuclear transfer (see also Paragraphs 0115-0119 of the specification).

The Examiner makes much of the state of the art of somatic cell gene targeting at the time of the invention. In particular, the Examiner cites Sedivy & Dutriaux (1999, submitted as reference AT26 in Appellants Information Disclosure Statement filed

5/22/2002) to support the unpredictability of the art in the 6/16/06 office action, citing Sedivy for the proposition that "The reason gene targeting has been do difficult in somatic cells is that the absolute frequency of homologous recombination in somatic cells is some two orders of magnitude lower than in ES cells. To complicate matters further, frequencies of non-homologous recombination are typically very high." Appellants generally agree with the characterization of the state of the art of homologous recombination prior to the present invention.

Yet, as noted above, gene targeting technology is the same for all cells regardless of whether the cell is a somatic cell, a stem cell or any other cell. Appellants took an interest in somatic cells, although others in the field were working with ES cells. In Examples 1-7 of the pending application, the inventors were able to demonstrate relatively high frequencies (6-66%) of successful gene targeting of 4 loci (placement of a neo marker gene at the COLIA-1 locus, placement of the AATC2 transgene at the COLIA-1 locus, knockout of porcine alpha-1,3-galactosyltransferase, knockout of ovine beta-lactoglobulin, knockout of bovine beta-lactoglobulin) in 4 different somatic cell types (ovine fetal fibroblasts, porcine fetal fibroblasts, bovine fetal fibroblasts, ovine epithelial cells). The present invention demonstrated that gene targeting in somatic cells actually can occur at relatively high frequencies, at least equal to that of the frequencies achieved in ES cells.

A brief review of the file history shows that the Examiner had previously focused rejections in prior office actions on the basis that it was not possible to screen for targeted integration events in somatic cells. The Appellants have overcome this rejection, as acknowledged by the Examiner in the 6/16/06 Final office action, "Specific targeting of an endogenous location in a somatic cell is not found to be unpredictable" (page 6); "Applicants have provided Declarations stating that using FACS- and PCR- based technologies are routine to screen for homologous recombination events (page 8)."

The Examiner also cited to Thomson and Polejaeva & Campbell to assert that premature senescence often occurs in gene targeted cells, which makes it difficult to confirm a targeting event in somatic cells. The data presented in the present application clearly establishes, however, that not only is it possible to achieve high efficiency

targeting and selection of targeted somatic cells, but that these cells remain competent and can be used to generate viable, cloned animals.

Thus, Appellants submit that the evidence of record establishes that at the time of filing, one skilled in the art would not require be required to engage in undue experimentation to specifically target a particular gene in the genome of a somatic cell, modify this particular gene and then identify and select the recombinant cells. Appellants further submit that the Examiner has failed to meet her burden to establish non-enablement.

2. Nuclear Transfer

Somatic cell nuclear transfer does not require undue experimentation

The Examiner maintains that nuclear transfer, and in particular somatic cell nuclear transfer, is unpredictable and thus, non-enabled. As detailed in the 6/16/06 Final Office Action, the Examiner states that "nuclear transfer, as a method may be well known, but determination of the state of the art at the time of filing, and consideration of the working examples of the specification, provide sufficient evidence that this technology is unpredictable with regard to what cell types to use as donors ("donor cell"), particular oocytes ("recipient cell") and the subsequent activation and further development of the NT unit to form a live born offspring ("development").....the full scope of the claims require successful transfection of the somatic cell, the selection of the transfected somatic cell (addressed above), the development of the resultant NT unit to form a live born animal, and these steps as a whole are not found to be enabling, for reasons set forth previously (see also Fulka, Oback, Campbell, Tian, Li and McEvoy, cited previously)." Appellants address each of these specific rejections, in turn, below.

A brief review of the file history reveals that the Examiner supports the non-enablement rejection with post-filing publications that focus on academic insights into the cloning process and possible ways to improve the overall efficiency nuclear transfer by analyzing the underlying molecular mechanisms. In general, the Examiner and Appellants seem to agree that cloning is an inefficient process that works some percentage of the time and with a technically feasible frequency to achieve success. The Examiner and Appellants disagree, however, of the significance of that inefficiency. The

Appellants are in the business of animal cloning to produce transgenic animals for use in xenotransplantation. In general, the Appellants are not interested in studying the mechanisms underlying the cloning process or ways to improve the cloning process. Rather, the Appellants have established a successful business model that employs the cloning process as it has existed for the past ten years, which takes into account the low efficiency of the technique, and have successfully cloned hundreds of animals (including genetically modified animals) to date. Thus, Appellants, through their own business model, clearly demonstrate that nuclear transfer is a routine technology that can be used successfully as the basis for a productive biotechnology business.

Appellants have submitted Declarations from those skilled in the art, Dr. Piedrahita and Dr. Ayares, both whom state that this invention is typical of the amount of experimentation required in the nuclear transfer art. In his Declaration (submitted May 11, 2006), Dr. Piedrahitia (a disinterested third party) notes that he regularly transfers 400 or more embryos to achieve a live birth via SCNT. And further, when utilizing certain cell types, he would transfer far more, i.e., up to 2000 or more embryos, and would consider that routine lab work. Although the numbers are large, the technique is the same, simply "repetition of standard techniques." As noted above, a disclosure may be enabling even though a considerable amount of routine experimentation is required to practice the invention. *See* PPG Indus. Inc. v. Guardian Indus. Corp., 75 F.3d 1558, 1564 (Fed.Cir.1996).

The Examiner has not provided any evidence to contradict the high level of experimentation acceptable in the cloning art. Rather, the Examiner provides references, which describes variables that may effect the overall efficiency of the process, or which describe the reasons why the process is inefficient, but provides no evidence to rebut the Appellants' statement, as supported by the Declarations, that such inefficiencies are clearly accepted in the art, which views them as entirely predictable and properly accounts for them.

(i) Somatic Donor Cells

A review of the file history reveals that there has been substantial debate between the Examiner and the Appellants regarding the use of any somatic cell as a nuclear donor cell.

Nuclear transfer technology has been practiced now for decades. Until 1997, undifferentiated embryonic cells were used as nuclear donors. In 1997, the successful cloning of sheep from adult mammary gland or fetal fibroblast cells (somatic cells) was announced (with the cloning of "Dolly"). Once begun, the progression to somatic cell cloning or nuclear transfer employing differentiated cells as the source of donor nuclei was rapid, initially involving differentiated embryonic cell cultures in sheep in 1996 and quickly thereafter, fetal or adult somatic cells in sheep, cow, mouse, goat, and pig. By 1999, sheep, cows and mice had been successfully cloned (see, for example, WO 97/07668; WO 97/07669; WO 98/39416; WO 98/30683; WO 98/07841; WO 99/01164; WO 99/01163; Wakayama et al. (1998). Nature, 394, 369-374; Wilmut et al (1997). Nature, 385, 810-813; Schnieke et al (1997), Science, 278, 2130-2133; submitted in Appellants' Information Disclosure Stated filed 5/22/2002). To date, at least thirty types of somatic donor cells have been used to clone at least fifteen different animals (see, "Somatic Cell Nuclear Transfer Cloning Efficiency" Paterson & Wilmut: www.roslin.ac.uk/public/webtablesGR.pdf; submitted as reference AG in Appellants' Information Disclosure Stated filed 7/25/2005).

The Appellants have presented two Declarations from those skilled in the art to establish that "there is no fundamental reason that prevents any somatic cell with a normal karyotype from acting as a nuclear donor..." (paragraph 11 of the Ayares declaration)"; "In 1999, I knew of no somatic cell that for theoretical or technical reasons could not be used as a supply of genetic material for cloning, or, in particular mammalian cloning (paragraph 15 of the Piedrahita Declaration)."

In the 6/16/06 Final Office Action, the Examiner acknowledged that the Appellants have provided "Declarations stating that cloning animals using any untransfected somatic cell is routine". Thus, Appellants believe that the two Declarations (Dr. Ayares' and Dr. Piedrahita's) submitted by persons of ordinary skill in the art have convinced the Examiner, who relied primarily on both the Oback and Tian references as the basis of the rejection, that this was the state of the art in 1999.

However, the Examiner further stated in the 6/16/06 Final Office Action that "it is noted that Applicants' arguments and the Piedrahita Declaration are directed to using untransfected somatic cell types for nuclear transfer. The claims require transfection of a

somatic cell, to be used in NT." The Appellants are frankly confused by this comment, it was clearly not routine in 1999 to use transfected somatic cells for nuclear transfer; this is the Appellants' invention, as discussed in detail above. The Examiner is effectively asking the Appellant to prove that their invention was routine at the time the application was filed, which of course is not appropriate.

(ii) Recipient cells

The Examiner has cited to Fulka et al (Theriogenology 2001 55 (6): 1373-1380) to support the rejection that only oocytes in metaphase II or telophase II can be used for successful nuclear transfer. Fulka et al. provides a review of cytoplasmic activities in oocytes that may be involved in reprogramming somatic nuclei after transfer into cytoplasts. Fulka et al. state that "current cloning schemes use at least three basic types of cytoplasts" (metaphase II oocytes used immediately, metaphase II oocytes aged in culture and telophase II oocytes). Fulka et al also state that mature metaphase II oocytes are the most commonly used source of cytoplasts, which is also discussed in the specification of the present invention. However, Fulka et al. does not suggest that other types of oocytes cannot be used for nuclear transfer, only that at least three are commonly used. In fact, Miyoshi et al. (BMC Developmental Biology (2001) 1:12, submitted as reference BI in Appellants IDS submitted 7/14/2004), teaches the use of metaphase I stage oocytes for nuclear transfer. Thus, one skilled in the art of nuclear transfer would be able to select an appropriate oocyte, two cell embryo or zygote as a recipient cell to producing a viable nuclear transfer unit.

(iii) Development

The Examiner has maintained rejections regarding the unpredictability of the development of cloned animals, as supported by the post-filing publication of Campbell et al. (Reprod. Dom. Anim., 40: 256-268 (2005)); Li et al. (Reprod. Bio. & Endocrin., I(84)1-6 (2003); McEvoy et al. (Reprod. Supp., 61: 167-182); McEvoy et al. (Reproduction, 122: 507-508 (2001)), which are each addressed below. As noted above, these references are generally concerned with improving the efficiency of the process or in explaining the various reasons the process is inefficient. Neither, Appellants submit, provide adequate evidence that the process is non-enabled.

Campbell

The Examiner has argued that the unpredictability of SCNT is supported by the post-filing publication of Campbell et al., which the Examiner has cited to state: "Despite the apparent successes of the technology, the efficiency of development to live offspring has remained low and development abnormalities still occur."

Campbell et al. actually opens with the following statement which clearly supports enablement:

> It is now 8 years after the birth of Dolly, the first animal produced by nuclear transfer using a donor cell population established from an adult animal. During this time, the technique of nuclear transfer has been successfully applied to a range of mammalian species for the production of offspring using a plethora of donor cell types derived from both foetal and adult tissues. In addition, when coupled with genetic manipulation of the donor cells, transgenic offspring have been produced with a range of genetic modifications including gene knockouts and gene knockins.

It could not be more clear that Campbell, similar to the Declaration of Ayares and Piedrahita, acknowledges that somatic cell nuclear transfer works across a "plethora" of donor cell types, and that the Campbell article really addresses not whether SCNT is achievable with a range of donor cells, but instead attempts to increase the efficiency of the process. As noted above, any finding that a process could be more efficient should is inadequate to support non-enablement of that process, particularly where the relevant art (SCNT) accepts and accounts for such inefficiencies.

Li

The Examiner has further relied on Li et al. to support the enablement rejection. Li is another reference that focuses on aspects of the inefficiency of the NT process as a means to achieve an improvement. The Examiner points out that Li states that "Most cloned animals... fail to develop to term, and some of the surviving animals have shown abnormalities." That is not news in the nuclear transfer field, as expressed in Dr. Piedrahita's Declaration, who states that people working in the field of SCNT live with low efficiency results and expect them in the area of nuclear transfer and cloning (paragraph 16 of the Piedrahita Declaration, submitted 4/21/2006). Li also stated, however, that "Regardless of the inefficiencies of this process currently, morphologically

normal living animals have been produced in 10 species..." (pg. 2), and that "Our data....provide evidence that nuclear transfer, despite multiple disorders, can result in physiologically normal, fertile animals." (pg. 2) .

McEvoy

McEvoy et al. (Reprod. Supp., 61: 167-182) and McEvoy et al., (Reproduction, 122: 507-508 (2001)) have been cited by the Examiner as teaching that faulty or epigenetic reprogramming of the nuclear donor nucleus is responsible for the low efficiency and abnormal development said to be associated with cloning.

McEvoy concludes his Reproduction article with the following summary of his views:

> However, it is possible that we will only capitalize fully on the ever more sophisticated nuclear transfer and gene targeting technologies when unspectacular refinements to in vitro culture systems finally establish an environment that is hazard-free and that avoids perturbing the early regulation of mammalian development. Having already demonstrated what is possible, scientists must now realize that application of recent and remarkable research advances for the benefit of animal and human health will hereafter depend on minimizing, and preferably eliminating adverse consequences arising from in vitro manipulation of animal cells and embryos.

As with the other articles, McEvoy is focused on improving the efficiency of processes that have been shown to work or simply provides an explanation (i.e., epigenic reprogramming) for those inefficiencies. Neither is an adequate basis to support non-enablement.

3. The Invention as a Whole

The Examiner has not provided any evidence to support lack of enablement of the invention as a whole per se, but rather has based the enablement rejections on references that attempt to establish that either the nuclear transfer component or the homologous recombination component is unpredictable and/ or requires undue experimentation. In fact, there is no evidence available of which the Appellants are aware that the Examiner has cited that would support a lack of enablement for the invention as a whole per se. Quite to the contrary, the Appellants only know of reports of success of the method by at least nine independent research groups since the publication of the present invention.

Subsequent to the teachings of the present invention, at least nine independent research groups have successfully targeted five different genes in three different species using twelve different targeting constructs (Lai et al. (Science (2002) 295:1089-1092); Kolber-Simonds et al. (PNAS. (2004) 101(19):7335-40), Ramsoondar et al. (Biol Reprod (2003)69:437-445), Sharma et al. (Transplantation (2003) 75:430-436), Harrison et al. (Transgenic Research (2002) 11:143-150) & Harrison et al. (Cloning and Stem Cells (2004) 6:327-331), Takahagi et al. (Molecular Reprod. Dev. (2005) 71:331-338), Dai et al. (Nature Biotechnology (2002) 20: 251-255) & Phelps et al. (Science (2003) Jan 17;299(5605):411-4), Denning et al. (Nature Biotechnology (2002) 19:559-562), Wells (Nature Biotechnology (2002) 19:529-530), Sendai et al. (Transplantation (2003) 76:900-902), Sendai et al. (Transplantation (2006) 81:760-766), Kuroiwa et al. (Nature Genetics (2004) 36:775-780), Richt et al. (Nature Biotechnology (2007) 25:132-138), Shen et al. (Molecular Reprod. Dev. (2006) 74:428-434)) to produce viable genetically modified animals. Six of these groups have targeted the alpha-1,3-galactosyltransferase (alpha-1,3-GT) gene in pigs, which is detailed below to provide an understanding of the breadth of strategies that have been used to accomplish the production of genetically modified cloned animals since the present invention was published.

The six groups include: Immerge Biotherapeutics, Inc. in collaboration with the University of Missouri, Alexion Pharmaceuticals, Nextran Incorporated, BresaGen Limited, The Animal Engineering Research Institute in Japan, and Revivicor, Inc. Each group designed its own targeting vector, which contained varying lengths (6.2-21 kb) and regions of homology to the endogenous target locus; different target loci (exons 4, 9); varying gene targeting strategies (promoter trap, positive-negative selection); and different genetic backgrounds (miniature and large white commercial swine, existing transgenic swine) as a source of donor cells to produce healthy pigs with a targeted disruption of the alpha 1,3 GT gene and reported similar gene targeting efficiencies in somatic cells. Using six different vectors, each of these groups targeted the alpha 1,3 GT gene in somatic cells, and used them as nuclear donors to produce viable genetically modified piglets.

Specifically, Revivicor, Inc. targeted exon 9, the functional coding region of the alpha 1,3 GT gene, using a promoter trap vector, with approximately 6.2 kb of homology to the endogenous gene. Immerge Biotherapeutics used a promoter trap vector, with a 21kb region of homology, also targeting exon 9, in miniature swine. The Alexion group targeted exon 4 of the alpha 1,3 GT gene, which encodes the translation initiation start codon using a promoter trap vector with 10.1 kb of homology. The Nextran group used positive negative selection methodology and a targeting vector with 7.1 kb of homology to target exon 9. The BresaGen group reported alpha 1,3 GT gene targeted porcine cells in 2002 and live born pigs in 2004. Their vector was a promoter trap targeting exon 4 and having 7.5 kb of homology. In 2005, the Japanese Animal Engineering Research Institute also produced alpha 1,3 GT gene knockout pigs using cells that already contained two other transgenes. Their vector was a promoter trap targeting exon 9.

This evidence of post-filing success demonstrates that multiple groups have been able to successfully produce genetically modified cloned animals as taught by the disclosure of the pending application.

4. Species/ Genus

The Examiner has maintained the rejection that the claims are not enabled because the claims recite transferring the nuclear transfer unit to a surrogate mother which is a suitable host and the Examiner argues that the specification does not provide guidance for a suitable host surrogate mother for the breadth claimed.

Whether or not a surrogate mother can carry an embryo of another species to term is an inherent limitation known to those skilled in the art. There are various combinations known to those skilled in the art, examples were detailed in Appellants' Response to Office Action dated 7/14/04 (page 19) and include: Alapacas gestated in the womb of llamas; african wildcats in the womb of domestic cats; Prezewalski's horse and Grant's zebra in the womb of domestic horses; horses in the womb of jack donkeys; European minks in the womb of hybrid minks. Thus, Appellants maintain that one skilled in the art has sufficient knowledge and can readily determine "suitable hosts" to act as surrogate mothers. As evidenced by Piedrahita's declaration, researchers in the field at the time the

application was filed were among the "elite" of the profession (paragraph 14 of the Piedrahita Declaration, submitted 4/21/2006).

5. Genotype/ Phenotype

The Examiner has continued to maintain rejections based on the unpredictability of the resulting phenotype of the transgenic animal, but has slightly modified the argument for the rejection over time. In the most recent, Final Office Action, mailed 6/16/06, the Examiner states "it is maintained that the specification fails to provide specific guidance for the breadth of producing any transgenic non-human animal whose genome comprises a modification at an endogenous locus by a gene targeting event, and thus, it would have required undue experimentation to predict the results achieved in any one host animal comprising and expressing a particular transgene, the levels of the transgenic product, the consequence of the product and the resulting phenotype."

Appellants continue to be perplexed by this rejection, the claims are directed to methods to produce genetically modified animals. Thus, the person skilled in the art practicing the claimed invention would be producing the transgenic animals themselves, which includes selecting a gene to modify and then producing the transgenic animal according to the methods of the present invention. Thus, the practitioner of the present invention would obviously be able to predict the resultant phenotype/genotype because they themselves would have designed the transgenic animal. Appellants also note that the pending claims do not include any limitations about the levels of the transgenic product, the consequence of the product and the resulting phenotype of the animal; the claims are directed to methods to produce the transgenic animals. Those skilled in the art can make and used the claimed invention to create any type of transgenic, cloned animal that they desire based on the teachings of the present invention.

D. Claim 133 is fully enabled

Claim 133 is enabled for all of the reasons discussed in detail above with reference to claim 131 and further, because claim 133 specifically recites certain steps involved in the accomplishment of successful nuclear transfer. The recitation of these steps limits the breadth of the claim relative to claim 131 and is further supported by the

specification. Moreover, claim 133 recites the identity of the recipient cell as an ooctye, two cell embryo or zygote, which limits the breadth of the claim relative to claim 131.

E. Claims 62 and 90 are fully enabled

Claims 62 and 90 are enabled for all of the reasons discussed in detail above with respect to claim 131, and further, because claims 62 and 90 specifically recite certain steps involved in the accomplishment of successful nuclear transfer. The recitation of these steps limits the breadth of the claim relative to claim 131 and is further supported by the specification. Moreover, claims 62 and 90 also recite the identity of the recipient cell, as an oocyte, two cell embryo or zygote, further limiting the claims relative to 131. With reference to claim 133, claims 62 and 90 require that the transgenic animal is successfully brought to term and results in a viable offspring, which the specification indeed describes by disclosing examples of the production of viable genetically modified sheep. In addition, as described above, there have been numerous examples of the post-filing success achieved by at least nine groups in producing viable genetically modified cloned animals. Claim 90 also includes the step of breeding the transgenic mammal to produce transgenic offspring from the transgenic mammal, further limiting the claim.

F. Claim 63 is fully enabled

Claim 63 specifies that the transgenic mammal of claim 62 is a transgenic sheep, cattle or pig. Claim 63 is enabled for all of the reasons referenced above with respect to claim 62, but further, because the claim is limited relative to claim 63 and because the Examples provided in the present application and the post-filing references described above, demonstrate successful production of genetically modified, cloned sheep, cattle and pigs.

G. Claims 65 and 99 are fully enabled

Claims 65 and 99 further limit the method of claims 62 and 90, respectively, by specifically reciting the type of genetic targeting event. Thus, in addition to the arguments above with reference to claims 62 and 90, claims 65 and 99 are further enabled because they are narrower in scope and additionally, because the specification provides

data in the Examples on the production of transgenic somatic cells and/or animals in which a transgene has been placed, genes have been modified and genes have been removed (specifically, a neo marker gene was placed at the COLIA-1 locus, the AATC2 transgene was placed at the COLIA-1 locus, porcine alpha-1,3-galactosyltransferase was inactivated, ovine beta-lactoglobulin was inactivated, and bovine beta-lactoglobulin was inactivated), in addition to transgenic sheep that contain a transgene placement. Further, there are numerous examples of post-filing success, as described above.

H. Claim 66 & 100

Claims 66 and 100 further limit the method of claims 62 and 90, respectively, by specifically reciting that the genetic targeting event results in inactivation of a gene. Thus, in addition to the arguments above as to the enablement of claims 62 and 90, the Examples of the present application describes the successful generation of ovine, bovine and porcine somatic cells in which two different endogenous genes were inactivated (porcine alpha-1,3-galactosyltransferase was inactivated, ovine beta-lactoglobulin was inactivated, and bovine beta-lactoglobulin was inactivated). In addition, there are numerous examples of post-filing success, as described above. Based on the disclosure, combined with the vast knowledge of one skilled in the art in producing constructs to inactivate genes (based on the generation of hundreds of "knockout" mice generated prior to 1999), these claims are fully enabled.

I. Claims 70-73 & 102-105

Claim 70-73 and 102-105 further limit the method of claim 62 and 90, respectively, by stating that the modification involves specific transgene placement of promoters, as well as the identity and function of the promoter, thereby narrowing the scope of the claims relative to claims 62 and 90. Moreover, the Examples of the pending application describe transgene placement including a neo marker gene was placed at the COLIA-1 locus, the AATC2 transgene was placed at the COLIA-1 locus.

J. Claims 75 and 106 are fully enabled

Claims 75 and 106 further limit the method of claims 62 and 90, respectively, by reciting that the modification places a marker gene at the endogenous locus in the nuclear genome, thereby narrowing the claim. Thus, in addition to the arguments above, the data in the present application describes the successful generation of ovine, bovine and porcine somatic cells in which a marker gene was inserted into 4 different loci (ovine COLT-1 locus, ovine BLG locus, porcine alpha GT locus, bovine BLG locus) to aid in the screening to detect successful homologous recombination events. The use of marker genes is a common tool to aid in the screening procedure and one skilled in the art would readily be able to employ any marker gene to achieve this purpose.

K. Claims 76-79 & 107-110 are fully enabled

Claim 76-79 and 107-110 further limit the method of claims 75 and 110, respectively, by reciting that the marker gene that confers resistance to neomycin, G418, hygromycin, zeocin, blasticidin and/or histidinol; the marker gene is HPRT, gpt, a visible marker gene and/or a gene that can be detected with a single chain antibody/hapten system; and/ or that the visible marker gene is GFP, thereby limiting the scope of the claim. The data in the present application describes the successful generation of ovine, bovine and porcine somatic cells in which either the neo or G418 marker gene was used as well as transgenic sheep containing the neomycin marker gene. In addition, the specification describes the use of specific marker genes (Paragraph 173) to aid in the screening to detect successful homologous recombination events.

L. Claims 82, 113, 121 and 122 are fully enabled

Claims 82, 113, 121 and 122 further limit claims 62 and/ or 90 by reciting that the genetic targeting event is mediated by lipofection, electroporation or transfection, thereby limiting the breadth of the claims. Thus, claims 82, 113, 121 and 121 are narrower in scope that claims 62 and 90. With respect to claim 121 and 122, as described in the specification, a survey of the extensive literature describing gene targeting indicates that transfection by electroporation is by far the method preferred in the art (see Paragraph

1026). With respect to claims 82, 113 and 122 the present application describes the use of transfection by lipofection in the Examples.

M. Claim 123 and 124 are fully enabled

Claims 123 and 124 further limit the method of claims 66 and 99, respectively, by reciting that the gene that is inactivated is α-1,3 galactosyltransferase, thereby limiting the scope of the claims. In addition to the arguments presented above, claim 123 is further enabled based on the teaching of the specification, the examples presented in the specification, and the post-filing success. The specification specifically discusses the inactivation, removal or modification of α-1,3 galactosyltransferase (Paragraph 0149) to aid in xenotransplantation. In addition, Example 6 demonstrates the successful generation of porcine somatic cells that were genetically modified to inactivate the α-1,3 galactosyltransferase gene. Further, the current Applicants, as well as five additional independent research groups, using the methods described in the present specification have to date used six different vectors to targeted the α-1,3 galactosyltransferase gene in somatic cells, and then used them as nuclear donors to produce viable genetically modified piglets.

N. Claim 125 is fully enabled

Claim 125 further limit the method of claims 62 or 90, by reciting that the endogenous locus is an immunoglobulin gene, limiting the breadth of the claim. In addition to the arguments presented above, claim 125 is further enabled based on the teaching of the specification (see, Paragraphs 0156 and 0163), and the post-filing success of the Appellants. Using the methods of the present invention, the Appellants have successfully gene targeted two different immunoglobulin loci (heavy chain and kappa light chain) and produced cloned piglets with immunoglobulin gene disruptions. In addition, researchers at Hematech, LLC and Kirin Brewery Company, Ltd, have also reported the production of cows by cloning using somatic cells, which had been specifically modified through genetic targeting to inactivate the immunoglobulin "mu" gene.

O. Claims 87 and 118 are fully enabled

Claims 87 and 118 limit claims the method of claims 62 and 90, respectively, by reciting that the somatic cell is an epithelial cell, a fibroblast cell or an endothelial cell, thereby limiting the breadth of the claim. In addition to the arguments presented above, Appellants specifically discuss the use of such cells in the specification and present data on the successful genetic modification of genes in fibroblast and epithelial cells in the Examples. Further, Dr. Ayares' Declaration includes data on the successful use of endothelial cells to produce cloned animals (paragraph 21 of the Ayares Declaration).

P. Claim 88-89 & 119-120

Claims 88-89 and 119-120 limit the method of claims 62 and 90, respectively, by reciting that the somatic cell is a G_0 cell or a G_0 cell obtained by serum starvation of a somatic cell, thereby limiting the breadth of the claim. In addition to the arguments presented above, claim 88-89 and 119-20 are further enabled by reference to the numerous examples of successful cloning of sheep, cows and mice successfully cloned using G_0 and G_0 cell obtained by serum starvation prior to 1999 (see, e.g., WO 97/07668; WO 97/07669; WO 98/39416; WO 98/30683; WO 98/07841; WO 99/01164; WO 99/01163; Wakayama et al. (1998). Nature, 394, 369-374; Wilmut et al (1997). Nature, 385, 810-813; Schnieke et al (1997), Science, 278, 2130-2133; submitted in Appellants' Information Disclosure Stated filed 5/22/2002).

(viii) CLAIMS APPENDIX

Claim 62. A method for producing a non-human transgenic mammal, the method comprising:

(a) modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event;

(b) transferring the modified nuclear genome of the somatic cell to a oocyte, two cell embryo or zygote which is capable of producing a viable nuclear transfer unit;

(c) activating the nuclear transfer unit thereby producing an embryo;

(d) transferring the embryo to a surrogate mother which is a suitable host; and

(e) allowing the embryo to develop to term, thereby producing a non-human transgenic mammal.

Claim 63. The method of claim 62, wherein the transgenic mammal is a transgenic sheep, cattle or pig.

Claim 65. The method of claim 62, wherein the genetic targeting event results in removal of a gene, modification of a gene, upregulation of a gene, gene replacement or transgene placement.

Claim 66. The method of claim 62, wherein the genetic targeting event results in inactivation of a gene.

Claim 70. The method of claim 62, wherein the modification comprises placing a promoter adjacent to an endogenous gene in the nuclear genome.

Claim 71. The method of claim 70, wherein the promoter is a collagen gene promoter.

Claim 72. The method of claim 70, wherein the promoter is a milk protein gene promoter.

Claim 73. The method of claim 70, wherein the promoter directs expression of at least one gene in fibroblast cells.

Claim 75. The method of claim 62, wherein the modification comprises placing a marker gene at the endogenous locus in the nuclear genome.

Claim 76. The method of claim 75, wherein the marker gene is a gene that confers resistance to a drug.

Claim 77. The method of claim 76, wherein the gene that confers resistance to a drug is selected from the group consisting of neomycin, G418, hygromycin, zeocin, blasticidin and histidinol.

Claim 78. The method of claim 75, wherein the marker gene is selected from the group consisting of HPRT, gpt, a visible marker gene and a gene that can be detected with a single chain antibody/hapten system.

Claim 79. The method of claim 78, wherein the visible marker gene is GFP.

Claim 82. The method of claim 62, wherein the genetic targeting event is mediated by lipofection.

Claim 87. The method of claim 62, wherein the somatic cell is an epithelial cell, a fibroblast cell or an endothelial cell.

Claim 88. The method of claim 62, wherein the somatic cell is a G_0 cell.

Claim 89. The method of claim 88, wherein the G_0 cell is obtained by serum starvation of a somatic cell.

Claim 90. A method for producing transgenic offspring from a transgenic mammal, the method comprising:

(a) modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event;

(b) transferring the modified nuclear genome of the somatic cell to an oocyte, two cell embryo or zygote which is capable of producing a viable nuclear transfer unit;

(c) activating the nuclear transfer unit thereby producing an embryo;

(d) transferring the embryo to a surrogate mother which is a suitable host;

(e) allowing the embryo to develop to term, thereby producing a non-human transgenic mammal; and

(f) breeding the transgenic mammal to produce transgenic offspring from the transgenic mammal.

Claim 99. The method of claim 90, wherein the genetic targeting event results in removal of a gene, modification of a gene, upregulation of a gene, gene replacement or transgene placement.

Claim 100. The method of claim 90, wherein the genetic targeting event results in inactivation of a gene.

Claim 102. The method of claim 90, wherein the modification comprises placing a promoter adjacent to an endogenous gene in the nuclear genome.

Claim 103. The method of claim 102, wherein the promoter is a collagen gene promoter.

Claim 104. The method of claim 102, wherein the promoter is a milk protein gene promoter.

Claim 105. The method of claim 102, wherein the promoter directs expression of at least one gene in fibroblast cells.

Claim 106. The method of claim 90, wherein the modification comprises placing a marker gene at the endogenous locus in the nuclear genome.

Claim 107. The method of claim 106, wherein the marker gene is a gene that confers resistance to a drug.

Claim 108. The method of claim 107, wherein the gene that confers resistance to a drug is selected from the group consisting of neomycin, G418, hygromycin, zeocin, blasticidin and histidinol.

Claim 109. The method of claim 106, wherein the marker gene is selected from the group consisting of HPRT, gpt, a visible marker gene and a gene that can be detected with a single chain antibody/hapten system.

Claim 110. The method of claim 109, wherein the visible marker gene is GFP.

Claim 113. The method of claim 90, wherein the genetic targeting event is mediated by lipofection.

Claim 118. The method of claim 90, wherein the somatic cell is an epithelial cell, a fibroblast cell or an endothelial cell.

Claim 119. The method of claim 90, wherein the somatic cell is a G_0 cell.

Claim 120. The method of claim 119, wherein the G_0 cell is obtained by serum starvation of a somatic cell.

Claim 121. The method of claim 62 or 90, wherein the genetic targeting event is mediated by electroporation.

Claim 122. The method of claim 62 or 90, wherein the genetic targeting event is mediated by transfection.

Claim 123. The method of claim 66, wherein the gene that is inactivated is α-1,3 galactosyltransferase.

Claim 124. The method of claim 99, wherein the gene that is inactivated is α-1,3 galactosyltransferase.

Claim 125. The method of claim 62 or 90, wherein the endogenous locus is an immunoglobulin gene.

Claim 131. A method for producing a non-human transgenic mammal, the method comprising:

(a) modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event;

(b) accomplishing successful nuclear transfer to produce the non-human transgenic mammal.

Claim 133. A method for producing transgenic offspring from a transgenic mammal, the method comprising:

(a) modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event;

(b) transferring the modified nuclear genome of the somatic cell to an oocyte, two cell embryo or zygote which is capable of producing a viable nuclear transfer unit;

(c) activating the nuclear transfer unit thereby producing an embryo;

(d) transferring the embryo to a surrogate mother which is a suitable host;

(e) allowing the embryo to mature.

(ix) EVIDENCE APPENDIX

A. Declaration of David L. Ayares, Ph.D. dated June 23, 2005 (see attached)
B. Declaration of Jorge A. Piedrahita, Ph.D. dated April 21, 2006 (see attached)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/080,713	Confirmation No.: 9155
Applicant	:	Alan Colman et al.	
Filed	:	February 25, 2002	
TC/A.U.	:	1600/1632	
Examiner	:	Thaian N. Ton	
Title	:	Method of Preparing a Somatic Cell for Nuclear Transfer	
Docket No.	:	10758.105015	
Customer No.	:	20786	

June 23, 2005

DECLARATION OF DAVID L. AYARES, Ph.D.

1. My name is David L. Ayares. I am a cell biologist and embryologist working primarily in the field of nuclear transfer and genetic modification of donor cells to develop transgenic cloned animals for human therapeutic applications.

2. I attended Purdue University where I obtained a B.S. in Microbiology in 1982. I then obtained a Ph.D. in mammalian genetics from the University of Illinois Medical Center in 1987 where I analyzed the molecular biology of homologous recombination and DNA mismatch repair in mammalian cells. Following my graduate studies, I was a Postdoctoral Fellow in the Department of Biology at the Massachussetts Institute of Technology where I studied the molecular biology of protein degradation in mammalian cells.

3. Since 1990, I have been working in the biotechnology/ pharmaceutical industry. I worked at Abbott Laboratories as a Senior Research Cell/Molecular Biologist where I directed transgenic animal research and focused on the use of homologous recombination to generate genetic modifications in human cell lines and mouse embryonic stem cell lines until 1995. From 1995-1997, I worked at Baxter Healthcare as a research scientist in the gene therapy division focusing on gene delivery for gene therapy and the development of adenovirus-based vector systems, as well as novel helper cell lines, and transgenic animal models for *in vivo* gene therapy.

4. In 1997, I joined PPL Therapeutics, Inc., first as the Head of Molecular and Cell Biology and then as the Chief Operating Officer and Vice President of Research, where I directed all administrative and scientific aspects of the US Division of PPL Therapeutics. I managed the research of a multi-disciplinary team of scientists, focused on the development of novel vectors, cell lines, and transgenic animals (including mice, rabbits, pigs and cows) for applications in xenotransplantation, stem cells, and the production of therapeutic human proteins in milk.

5. I am currently the Chief Executive Officer and Chief Scientific Officer of Revivicor, Inc. (formerly PPL Therapeutics, Inc.), where I direct all administrative and scientific aspects of the company. Revivicor focuses on regenerative medicine including the production of genetically modified animals to provide human compatible cells, organs and tissues for use in human transplant surgery (xenotransplantation), as well as the production of therapeutic fully human polyclonal antibodies derived from genetically modified animals for a variety of infectious disease applications.

Somatic Donor Cells

6. I have read and I understand the article Oback B & Wells D. (2002) Donor cells for Nuclear Cloning: Many are Called, but Few are Chosen. Cloning Stem Cells 4(2): 147-168). I have in particular studied the data presented in Table 1 of the Oback paper.

7. Table 1 lists experimental cloning efficiencies of a range of somatic donor cells. Several of the somatic cell types have a reported "0" cloning efficiency. From that information, I understand that the Examiner has concluded that not all somatic donor cells can be used in all animal species to produce a live animal. I also understand that the Examiner suggests that "the state of the art clearly teaches that the donor cells to be used in somatic NT methods are neither predictable nor routine for different species."

8. Based on my experience in this field, I disagree with the Examiner's interpretation of the Oback paper. I know of no somatic cell that cannot be cloned as long as it has a normal karyotype.

9. The concept of cloning efficiency is a reflection of the ratio of number of attempts versus the number of successes (i.e, viable clones obtained). It illustrates a very well known and simple observation that certain somatic cells produce more viable embryos after transfer than others. Since some somatic cells produce viable embryos with a lower efficiency than others, when it is desired to use such lower efficiency cells, more embryos should be transferred.

10. The general technique of nuclear transfer is well known. When using a new donor cell type for somatic cell nuclear transfer, the worker simply has to recognize that it is a numbers game, and one must repeat the experiment using the well known techniques until success is achieved.

11. There is no fundamental reason that prevents any somatic cell with a normal karyotype from acting as a nuclear donor. If a viable embryo is not obtained, then the researcher simply needs to conduct more embryo transfers until viability is achieved. Repeating the experiment enough times to achieve success does not require a special or extra technique, it simply requires that you carry out the experiment more times. It can routinely require hundreds of embryo transfers to produce a viable cloned animal.

12. In my experience, while in most cells it is sufficient to transfer between 200-500 embryos to obtain a viable offspring, on two occasions we have carried out as many as 2000 embryo reconstructions to obtain a viable cloned offspring. The transfer of as many as 2000 embryos can take as little as two weeks to perform on a routine basis.

13. Indeed, Table 1 of Oback reports the cloning efficiency of fibroblast cells. Fibroblasts have been the most commonly used donor cell for nuclear transfer. Oback reports a cloning efficiency for fibroblasts of between 0.05% and 1.2%. A cloning efficiency of 0.05% represents 1 viable clone (i.e. live birth) per 2000 embryos transferred.

14. The cloning efficiency data generated by Oback based on the information presented in Table 1 are inaccurate due to the phenomenon of sampling error. When a sample size is insufficiently small, it does not represent the population from which it is taken and can thus lead to false negative results.

15. Table 1 omits the most important numbers, which are the number of embryos transferred to surrogate mothers in each experiment. The number of embryos transferred is a critical number because it relates directly to cloning efficiency. As discussed above, cloning efficiencies of 0.05% have been reported, which represents 2000 embryos transferred for 1 live birth. Table 1 provide "blastocyst" numbers as percentages calculated on the basis of numbers of reconstituted NT embryos. The Table also presents "implantation" numbers as percentages calculated on the basis of numbers of embryos transferred.

16. I have referred to the citations in the Table and calculated the actual number of embryos transferred. In particular, references 8 (Wakayama and Yanagimachi (2001). Molecular Reproduction and Development, 58, 376-383) and 15 (Wakayama *et al.* (1998). Nature, 394, 369-374) of Table 1 provide data for murine mature sertoli cells, murine lymphoctytes, murine macrophages, murine leukocytes and murine neurons as donor cells, for which the cloning efficiency is reported in Oback as "zero". My calculation of the number of embryos transferred for each of those cell types is listed below.

Tissue Origin	*Cell Type*	*Species*	*Embryos Transferred*	*Reference*
Adult				
Testis	Mature Sertoli	Mouse	59 (total #)	8
Thymus	Lymphocyte	Mouse	0	8
Peritoneal Cavity	Macrophages	Mouse	52/25 (total #: female/ male)	8
Spleen	Leukocytes	Mouse	11/8 (total #: female/ male)	8
Brain	Neuron? Glia?	Mouse	46 (total #)	15

17. As evident in the above table, the cloning efficiencies for the cell types listed were calculated based on 0-75 total embryo transfers per cell type. Thus, the sample number was too small to draw any conclusions on the "clonability" of these cell types.

18. Indeed, for some cell types used in studies cited in Table 1 cloning efficiency was originally reported as zero, however, more recent publications have clearly demonstrated that viable clones are produced.

19. For example, in Table 1, murine neuronal cells were reported to have a "0" cloning efficiency. However, Yamazaki et al reported that when neural cell nuclei were transferred into enucleated oocytes, 5.5% of the reconstructed oocytes developed into normal mice (Yamazaki, et al, Assessment of the developmental totipotency of neural cells in the cerebral cortex of mouse embryo by nuclear transfer. *Proceedings of the National Academy of Sciences*, November 20, 2001, Vol 98, pages 14022-14026).

20. Also in Table 1, murine lymphocytes from the thymus and leukocytes from the spleen were reported to have a "0" cloning efficiency. However, Hochedlinger & Jaenisch (*Nature* 2002 Feb 28 :Monoclonal mice generated by nuclear transfer from mature B and T donor cells" 415(6875):1035-8: Epub 2002 Feb 10) reported the generation of monoclonal mice by nuclear transfer from mature T cells (lymphocytes from the thymus) and B cells (leukocytes from the spleen). In particular, the authors state "This is an unequivocal demonstration that a terminally differentiated cell can be reprogrammed to produce an adult cloned animal."

21. As an example of cloning new, as yet never tried, cell types, we recently set out to clone pigs using a donor cell type, endothelial cells, which had never been used for cloning. Our first round of new experiments resulted in the successful production of cloned pigs from endothelial cells. We transferred 341 embryos into one surrogate mother. This transfer resulted in pregnancy and delivery of three healthy piglets on day 119 of gestation. Thus, our cloning efficiency for endothelial cells was 0.9%, which is within the range reported for other cell types. This represents a typical strategy that one skilled in the art would employ to clone a new cell type.

Homologous Recombination

22. Homologous recombination is a natural event that occurs in all cells. Homologous recombination underlies many biological pathways. The recombination machinery has been well conserved throughout evolution as an essential component of cell survival. In cells, homologous recombination is a DNA maintenance pathway that protects chromosomes against damage affecting both DNA strands, such as double strand breaks or interstrand crosslinks. In addition to its maintenance role, homologous recombination underlies numerous other biological events. For example, it is involved in meiotic crossovers, which are responsible for the rearrangement of alleles, as well as necessary for proper chromosome segregation. It is also responsible for mating type switching, epitope class switching, mutations, translocations, gene rearrangements and evolution in cells.

23. Homologous recombination is the leading technology in rational genome engineering. Many fundamental discoveries in biology have taken place with the help of homologous recombination. Scientists have exploited the cell's endogenous machinery to use homologous recombination to manufacture site directed insertions, deletions or replacements of DNA in a living organism for the past 20 years.

24. Therefore, all cells can successfully undergo homologous recombination because it is an essential mechanism required for all viable cells.

25. During the interview with the Examiners, the issue was raised regarding whether cells other than fibroblasts could be targeted since other cell types may not have equivalent proliferative potential. As discussed with the Examiners, it was clearly feasible as of the filing date of the present application to obtain and screen for homologous recombination in cell types that do not have high proliferative potential. This fact was first demonstrated in 1989 by Zimmer and Gruss (Nature 338: 150-153) and 1990 by Jasin et al (Genes & Development (1990) 4: 132-166).

26. Zimmer & Gruss described a rapid polymerase chain reaction (PCR)-based screening method to identify homologous recombination events in mouse embryonic stem cells without prolonged culture and cell expansion. This paper was hailed as a significant advance since, at that time, relatively few mouse embryonic stem cell lines could be cultured long term without undergoing differentiation. This was problematic since one could identify homologous recombination events after prolonged culture but since the embryonic stem cells had differentiated they were not useful for production of germ line chimeras. Zimmer's PCR assay (in which a diagnostic PCR product is amplified using one primer within a marker gene within the targeting vector, and a second primer located in the genomic target sequence outside the region of homology), did not require growth of embryonic stem cell clones from single colonies to one million cells (as required prior to the Zimmer publication) but rather could be used to identify homologously recombined clones as soon as they had formed.

27. The use of Fluorescent Activated Cell Sorting (FACS) to rapidly detect homologous recombination events in transfected cells has facilitated genetic targeting in all cell types. The Jasin paper (Genes & Development (1990) 4: 132-166) was a fundamental publication that established the importance of this strategy of selection for gene targeting. The Jasin paper reported a 700-fold enrichment for homologous versus nonhomologous integration events by using FACS to select the targeted cells.

28. Therefore, as early as 1989, several methods, including PCR-based and FACS-based techniques, were known to those skilled in the art, which could be used to detect targeted integration events after homologous recombination within 3-5 days. These methods allowed one to identify targeted clones, in a variety of cell types, without prolonged in vitro growth and expansion. It is clearly not a pre-requisite that cells to be used for homologous recombination and subsequent nuclear transfer must have high or even moderate proliferative potential.

29. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing thereon.

6/22/05
Date

David L. Ayares, Ph.D.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/080,713	Confirmation No.: 9155
Applicant	:	Alan Colman et al.	
Filed	:	February 25, 2002	
TC/A.U.	:	1600/1632	
Examiner	:	Thaian N. Ton	
Title	:	Method of Preparing a Somatic Cell for Nuclear Transfer	
Docket No.	:	10758.105015	
Customer No.	:	20786	

April 21, 2005

DECLARATION OF JORGE A. PIEDRAHITA, Ph.D.

1. My name is Jorge A. Piedrahita. I am Professor of Genomics in the College of Veterinary Medicine at North Carolina State University (NCSU), where my research is focused on the production of transgenic animals by somatic cell nuclear transfer (SCNT) and on the development of methods of homologous recombination in somatic cells.

2. I attended the University of British Columbia, Vancouver, where I obtained a B.Sc. in Agriculture in 1981. In 1984, I obtained a M.Sc. in Animal Sciences from the University of California, Davis, where I was also awarded a Ph.D. in Cell and Developmental Biology in 1998. Following my graduate studies, I was a Postdoctoral Fellow in Molecular Genetics at the University of North Carolina, Chapel Hill.

3. Since completion of my post-doctoral research in 1991, I have spent my career in academia.

4. From 1991-1997, I was an Assistant Professor in the Department of Veterinary Sciences and the Department of Veterinary Anatomy at Texas A&M University (TAMU). In 1997, I was promoted to Associated Professor in both Departments, and appointed Co Director of the Transgenic Core Facility at TAMU. In addition, I was named as a Member of the Intercollegiate Faculty of Genetics.

5. In 1998, to further my interest in SCNT, I spent several weeks working in the laboratory of Dr. Randy Prather at the University of Missouri-Columbia. Dr. Prather is a leading researcher in cloning, and is considered an expert in SCNT.

6. In 1999, I spent sixth months as a Visiting Scientist/Fogarty Fellow in Hamilton, New Zealand to further my understanding and skill in SCNT.

7. Upon my return from New Zealand, I established the SCNT program at TAMU in 1999.

8. From 1999-2002, I served as Associate Director of the Center for Animal Biotechnology and Genomics at TAMU. During that time, I was also appointed and served as Vice-Chair of the Interdisciplinary Genetics Program and Chair of the Professional Program in Biotechnology.

9. I am presently a Professor of Genomics in the College of Veterinary Medicine of North Carolina State University (NCSU). I also serve as the Interim Director of the Center for Comparative Medicine and Translational Research at NCSU.

10. My research at NCSU is directed to the cloning of transgenic animals by SCNT, and in particular, cloning of transgenic cattle and swine. My research is also directed to the study of methods for homologous recombination in cultured somatic cells.

11. I am the author of over 70 peer-reviewed publications, the inventor of several pending and issued patent applications, and have received numerous awards in the field.

12. I have been given a copy of the following documents: (i) U.S. Patent Application No. 10/080,713 ("Method of Preparing a Somatic Cell for Nuclear Transfer"); (ii) the Office Action dated January 24, 2005; (iii) the Response to Office Action (RCE) dated July 25, 2005; and (iv) the Ayares Declaration and supporting references.

13. I have read and I understand the documents referenced in paragraph 12.

Somatic Cell Donors

14. I understand that this matter involves the state of research in 1999 in the field of nuclear transfer as well as homologous recombination. I was active in the field of nuclear transfer and homologous recombination in 1999. The researchers in these areas at that time, and today, are highly skilled and highly educated. My general observation is that scientists acting independently in these fields in 1999 had a Ph.D. degree and significant work experience in sophisticated laboratory molecular biology techniques. They were among the "elite" of the animal veterinary research profession.

15. I understand that one of the issues raised by the Examiner is whether those of us in the field in 1999 would have thought that it was unpredictable which somatic cells could be used in somatic cell nuclear transfer to produce a clone. My response is that it was understood and well accepted by those of us working independently in the field at the time that the genetic material from any somatic cell could be used in somatic cell nuclear transfer. It was also understood and discussed that the more differentiated the cell, the less efficient the reprogramming might be, however, that was expected and accounted for. It was generally observed that some somatic cells had a higher cloning efficiency than others. However, I, as well as others, distinguished cloning efficiency from cloning ability. Low cloning efficiency simply meant that more transfers were required to achieve a success. In 1999, I knew of no somatic cell that for theoretical or technical reasons could not be used as a supply of genetic material for cloning, or in particular, mammalian cloning.

16. In 1999 I was not aware, and today am still not aware, of any publications or disclosures that reported that a certain somatic cell cannot be used for somatic cell nuclear transfer,

and in particular mammalian cell SCNT. A number of articles discussed the efficiency of SCNT in 1999, but none that I can recall made a statement that SCNT could not be successfully accomplished with any somatic cell using standard techniques in the industry. Those of us in the field of SCNT live with low efficiency results and expect them in the area of nuclear transfer and cloning. We are typically both time and resource constrained, and therefore use the somatic cells that are known to produce the highest numbers of live offspring simply out of convenience. That, however, should not and can not be interpreted as an implication that genetic material from other somatic cells can not be used.

17. In my own work, I typically use fetal fibroblasts for SCNT. I have also used other somatic cell types including follicular cells, but I generally use fetal fibroblasts simply because they are easy to work with and the goal of my work is to efficiently produce live offspring. I regularly transfer about 400 embryos to achieve a live birth via SCNT even with this highly efficient somatic cell.

18. If in 1999 or today, I were asked to carry out nuclear transfer to achieve a clone using a cell other than a fetal fibroblast, I would transfer far more, e.g., up to 2000 or more embryos, and would consider that a routine part of the lab work. If asked to clone with a difficult or inefficient cell line, I would commit more time and resources to the project as needed to achieve success. The transfer of a larger number of embryos naturally involves more work and cost, but can be carried out by repetition of standard techniques.

19. I have read and I agree with the Declaration of David Ayares. I am of the opinion that Dr. Ayares' Declaration represents the views of those working independently in the field of nuclear transfer in 1999. There is no question that the concept of cloning efficiency is simply a reflection of the ratio of number of attempts and not any indication of the lack of clonability of the cell. Again, there is no good scientific rationale to support the position that any given somatic cell cannot be cloned.

20. I would also like to comment on the Oback and Wells paper. I know David Wells, the senior author on the Oback paper, and as stated above, in 1999, I spent six months as a Visiting Scientist/Fogarty Fellow in Hamilton, New Zealand in the Wells laboratory to further my understanding and skill in SCNT. After I left, Oback worked and trained under the direction of David Wells. Wells does not state in the paper that there are somatic cells that are unclonable. His emphasis is on cloning efficiency. Further, I, as well as others, would know that Table 1 of his paper describes populations of cell experiments that are too small to reach any definitive conclusion on an accurate cloning efficiency of the listed somatic cells. The Oback and Wells paper focuses on differences among donor cells. The factors discussed therein are simply explanations, or rationalizations, of the differences in cloning efficiencies, not ultimate clonability. I further note that in the last sentence of the article (page 162), Wells states that "Cloning from adult neurons presents a considerable challenge and even though initial attempts failed (Wakayama et al , 1998), it cannot be considered biologically impossible". This is consistent with my statements above that there is no good scientific rationale to support the position that any given somatic cell cannot be cloned.

Homologous Recombination

21. I have been told that that the Examiner has stated that "although homologous recombination may be an essential event in all cells, this does not provide guidance with regard to the targeting of a particular homologous recombination event, and the subsequent selection of a particular cell." In fact, any cell that divides can be used to successfully carry out homologous recombination. A cell need not be highly proliferative, or even moderately proliferative, to undergo targeting by homologous recombination. Rather, homologous recombination requires only that a cell proliferate, regardless of rate.

22. The technique of homologous recombination is described in the standard textbook "Gene Targeting: "Gene Targeting: A Practical Approach. Alexandra L. Joyner, ed. Oxford University Press (1993).

23. I agree with Dr Ayares that it had been well described in the literature in 1999 to obtain and screen for homologous recombination events using PCR and FACS-based screening regardless of whether the genetically modified cells had high proliferative potential. These methods permit a researcher to detected targeted integration events without prolonged in vitro growth and expansion. The methods are also independent of what kind or kinds of cell is transfected. Therefore, it was irrelevant that the Zimmer and Gruss paper mentioned in Paragraph 26 of Dr. Ayares' declaration focused on ES cells or that the Jasin paper mentioned in Paragraph 27 of the Ayares Declaration focused on cultured COS-1 cells. The techniques, FACS and PCR, are equally applicable to all cells.

24. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing thereon.

4/25/06
Date

Jorge A. Piedrahita, Ph.D.